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A STUDY ON THE LIFE HISTORY OF A FLAGELLATE (*Crithidia melophagi*, n. sp.) IN THE ALIMENTARY TRACT OF THE SHEEP-TICK (*Melophagus ovinus*).*†

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SINCE the year 1902 insect flagellates have been a subject of much investigation in view of the possibility of their being developmental stages in the life cycle of the hemoflagellates. In a paper by Novy, MacNeal, and Torrey (1907), a most excellent résumé of the present knowledge concerning these parasites is given. It would therefore be entirely superfluous to review the literature in this connection.

This flagellate, to which I have given the name *Crithidia melophagi*, has been studied to some extent by E. Pfeiffer (1905). He claims the discovery of it for L. Pfeiffer about ten years before. As far as I am aware, no one else has given attention to it, though Dr. B. H. Ransom called to my notice the similarity between these forms and a flagellate which I (1907) discovered in the rat flea (*Ceratophyllus fasciatus*), about which more will be said later.

The ticks were removed from the sheep and examined, some immediately and others at various times after removal, so as to discover all possible stages and conditions of the parasites. To remove the stomach the posterior end of the abdomen was clipped with small scissors, and then, holding the tick on a slide by grasping the thorax with a pair of forceps, the contents of the abdomen were pressed out into a drop of salt solution placed on the slide. The pressure was effected by placing a needle on the anterior part of the abdomen and then drawing it toward the posterior end pushing the abdominal contents before it. The whole viscera including the oesophagus and rectum were thus squeezed out. With dissecting needles the digestive tract with its appendages was separated from the other viscera and straightened out, when it measured over an inch in length. This can all be done with the naked eye and without rupturing any portion of the tract. Sometimes the stomach wall is sufficiently clear for the moving flagellates within its lumen to be seen. If the ticks have not been long removed from the sheep, the anterior third of the stomach will be full of fresh blood. The posterior two-thirds contains blood in all stages of digestion, the more posterior parts being darker and often almost black. In starved ticks the anterior third is empty, while the rest contains the dark disintegrated blood. The posterior portion is often so filled with parasites that there is very little space left for food material.

For sectioning, the tract was put into a fixing fluid, Hermann's, Merkel's, or Zenker's. Having straightened it out it was sectioned its whole length serially, and thereby the exact conditions of the parasites in all parts of the tract were determined. In other

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cases the stomach contents were teased in a drop of salt solution on a slide. By cutting the stomach into pieces and transferring these to other slides the flagellates from various regions were separated. They were either studied in the living condition or were used for permanent mounts. In making the stained preparations a small drop of the salt solution containing the parasites was placed on a slide and, before it became entirely dry, some killing fluid was dropped on, which fixed them to the slide. For this purpose various killers such as methyl alcohol, absolute alcohol and ether, Merkel's, Carnoy's, and Zenker's fluids were used. A hot solution of corrosive sublimate and 95 per cent alcohol, made by mixing two parts of 95 per cent alcohol with one part of a saturated aqueous solution of sublimate, was also used. While very good results were obtained by this method, perhaps the most satisfactory one is to invert the slides for ten minutes over the mouth of a jar containing formol, then allow them to dry. As the salt solution dries down the salt crystallizes so that the parasites are obscured. To remove the crystals the slides were then washed in hot water and again left to dry. Results obtained by fixing with heat or from allowing the preparations to dry before fixing the flagellates, as others have done, were always inferior to those obtained by the above methods. I tried also teasing the stomach contents in fluid from the body cavity and then smearing the mixture as in making blood films. A serious objection to this method is that some of the parasites are distorted and one cannot be sure whether he is studying a normal or a distorted form. This goes to confirm MacNeal's opinion (1904) and my own conviction that blood trypanosomes are often badly distorted in smears. The trypanosomes, I believe, suffer even more disaster from such treatment than these flagellates, because they are not so firm and rigid as the latter. The contact preparations were stained according to Wright, Giemsa, or with iron hematoxylin. Mayer's hemalum and iron hematoxylin were used for staining sections. As mounting media, balsam, damar, and cedar oil were used. The Zeiss apochromatic outfit, including the 2 mm. and 3 mm. oil immersion lenses, and the apochromatic condenser was used both with daylight and the Welsbach gas burner.

I follow Woodcock (1906) and Minchin (1908) in using trophonucleus for nucleus and kinetonucleus for blepharoplast.

These flagellates present as much variability in size and shape as the rat trypanosome during the period of multiplication. They vary from a very long slender form down to a small spindle-shaped flagellate or spherical mass. Thus, no general description will suffice but it is necessary to describe each form separately.

The long forms, flagellum included, measure about 30 to 45 μ long by 2 μ wide. Sometimes, however, they reach a length of 60 μ. On the other hand, the young dividing forms may be only 12 μ in length. The typical forms have the appearance of a thin band thickened and widened in the middle. They are often spirally twisted, sometimes to the extent of three or four turns. A well-developed undulating membrane is present (Plate 3, Figs. 1–4). It is formed by the extension of the anterior end of the body along the flagellum, generally to a point within 2 to 4 μ from its end. In some cases it extends clear to the end. The membrane is very thin near the end, while farther back it becomes thicker, especially along the side opposite the bordering flagellum. The flagellum does not pass into the body but proceeds along its thin membranous edge to the kinetonucleus which lies against the wall (Figs. 2, 3, and 6). That such is the case was plainly shown by staining a long time with iron hematoxylin and then destaining thoroughly, so that the flagellum stood out as a black line. The parasites not being smeared on the slide stood out so that their true form could be determined. Since the membrane is formed by the end and side of the body, there is no line of separation between the body and the membrane. The whole anterior end of the flagellate nearly back to the kinetonucleus takes part in the undulating movements. The posterior half of the body is stiff and firm. This rigidity is not due to a central axis such as described by Prowazek (1904) for his forms, nor to a thick wall. The wall is so thin that it can be demonstrated with no certainty neither in stained preparations nor by crushing the flagellates in the living state. The rigidity seems rather to be a property of the cytoplasm itself. Prowazek ascribes the cause for rigidity in his forms to this central axis which is nothing more than a couple of spirally twisted threads running longitudinally through the body. According to the laws of mechanics, it is impossible to say that the rigidity is due to such a structure. It would have been far more reasonable and plausible for him to have attributed the cause of rigidity to the cuticle (periplast) which, according to him, is so firm that it remains as a delicate sheath when the endoplasm is forced out by pressure.

There is but one flagellum present. Stained according to Giemsa or Wright this point was at times doubtful. But in the forms stained a long time with iron hematoxylin there was never any doubt, the flagellum standing out as a black line. Then, too, occasionally flagellates were found with the flagellum stripped from the membrane back nearly as far as the kinetonucleus. In these forms never more than one flagellum could be seen. This condition was described by Prowazek for his form, but he states that there are two flagella with a membrane stretched between them. He used Giemsa's stain, which gave doubtful results in my hands.

The trophonucleus is situated about the center or in the posterior third of the body. It is round or elliptical and varies in size. On the average it measures about 1.7 μ by 2.5 μ. When stained with the hematoxylins a central deeply staining granule is plainly visible. This was seldom seen when Giemsa's or Wright's stain was used. It is exceedingly brilliant when stained with borax carmine. The chromatin is in the form of a reticulum upon which a number of granules is to be found. They cannot be differentiated with the hematoxylins but are distinct when stained according to Giemsa. They vary in number from four to 16. The most frequent number is from seven to 11. When the number is small they are all arranged in the circumference of the trophonucleus, but when the number is large some occupy a more central position. Especially in the latter case, the trophonucleus is very similar to nuclei of multicellular animals. Since these granules occur in varying numbers and in resting nuclei, they should not be considered as chromosomes. I hold that they are comparable to the net-knots of false nucleoli in nuclei of higher forms. The central granule has a stronger affinity for borax carmine and the hematoxylins than does the other nuclear material. I have never been able to distinguish these structures in divisional stages, the nucleus appearing as a dense structureless mass.

The kinetonucleus lies in a transverse position anterior to or at the side of the trophonucleus. In profile view it is seen to lie against the edge of the body on the same side as the flagellum. Stained with iron hematoxylin or safranin it appears either as a straight rod with square or rounded ends or like two balls which have partially fused. But when stained according to Giemsa it often appears in addition to the above forms as an ellipse or as a crescent with the horns pointing anteriorly. This crescentic appearance is sometimes due to the fact that the posterior rim of the ellipse is heavily stained while the rest of the ellipse is faintly stained or not at all. The kinetonucleus is larger when stained according to Giemsa than when other stains are used. It appears from this fact that there is an outer area which stains only with Giemsa. At times it is nearly as large as the trophonucleus and presents much the same structure. It may or may not lie in a clear area. The flagellum at its juncture with the kinetonucleus often remains unstained, as in the trypanosomes. The cytoplasm is clear, finely granular, and reticulate. Generally small vacuoles, appearing in the living condition as refractive spots, are to be found mostly posterior to the trophonucleus. Between the vacuoles from four to 12 round or irregular granules, which stain with Giesma like the kinetonucleus, are present. They are deeply stained even when the trophonucleus is barely visible (Fig. 2). Iron hematoxylin, however, does not differentiate them. If they are chromatin it is strange that they are not differentiated at all with iron hematoxylin, but with Giesma stain even more readily than the trophonucleus. There are, however, other masses (probably chromatin) sometimes present that do stain with iron hematoxylin. No posterior diplosome could be found.

The posterior end may be abruptly pointed or bluntly rounded but seldom drawn out into a long tapering point (Figs. 1–4). Instead, it is often rounded off so as to form a large knob containing the trophonucleus or kinetonucleus, or both (Figs. 7, 8). In these cases the anterior end remains about the same as before. Others swell out in the region of the trophonucleus to form a ball-like structure in the middle of the body. Both of these types are very commonly found. Stiff spindle-shaped forms are also abundant (Fig. 5).

In sections these forms are found free in the lumen or attached in a single layer to the wall of the stomach by the anterior end. There are always as many attached as can possibly find room, no matter what the food condition may be. The remaining individuals are either free or are attached to free masses of epithelium. Sections of the malpighian tubes showed both long and short flagellate forms, some of which had traveled a considerable distance from the juncture of the tubes and the stomach.

The very long forms were never seen in process of division. In several cases, however, two trophonuclei were found. The long forms are to be regarded as adult or senile forms, which have originated by growth from the smaller types. This idea is confirmed by the fact that they are the first to die when the contents of the stomach are kept in salt solution. Some of the smaller forms remained alive more than five days at room temperature. Division, which is longitudinal, regularly takes place in the smaller forms (Plate 4, Fig. 9). This one figured is an exceptionally large divisional form. The kinetonucleus divides first and a new flagellum arises apparently by splitting off from the old one. The trophonucleus then divides and later the cytoplasm constricts. In no case was there any indication of mitosis. It is a striking fact that in spite of the enormous number of parasites present, division stages are very rare. However, in one tick gorged with dark-colored blood division forms were exceptionally abundant. Although they are scarce, some division stages are always present.

One is suprised at the vast number of parasites to be found by examining the stomach contents in the living condition after teasing the digestive tract in salt solution. They fill the digestive tube so full in the posterior two-thirds that it is a wonder how any food could pass through without crowding them out. Large rosettes with flagella centrally directed are present. It is a common thing to see two long slender forms agglutinate alongside each other with the flagella pointing in the same direction. Occasionally several are massed together in such fashion. There is no fixed law, however, causing them to agglutinate with flagella centrally directed, although this method is certainly the typical one. Two flagellates may, instead of coming together side by side with the flagella pointing in the same direction, agglutinate so that the flagella point in opposite directions. In this case the parasites tend to move in opposite directions, the flagellate end being the anterior end. The result is that they often slip past each other, so that only the posterior tips are in contact. Very frequently such couplets are seen, the stronger of them pulling the other through the fluid. Novy, MacNeal, and Torrey say that in agglutination rosettes the posterior ends are central in position as in the trypanosomes, but in division rosettes the flagella are centrally located as in the division rosettes of trypanosomes in culture. The former statement does not hold true in these forms. Whether the rosettes are formed by agglutination or division, they regularly have the flagella centrally directed.

In the intestine, which begins at the juncture of the malpighian tubes with the stomach, forms peculiar to this region were found. They correspond to the "formes grégariniennes" of *Herpetomonas subulata* as described by Léger (1904). Just posterior to the valve, which is at the point where the malpighian tubes enter the digestive tract, the intestine is much larger than the end of the stomach just anterior to the valve. It is funnel-shaped, the bowl being directed anteriorly. Lining the posterior wall of the valve which projects a short distance posteriorly into the funnel are several layers of parasites (Fig. 14). These forms are large, always rounded off at the posterior end. Those in the outside layer often measure as much as 20 μ in length. The individuals in the deeper layers are smaller. On the sides of this dilated portion of the intestine the parasites are smaller, yet very similar. Generally they are arranged in several layers, but there may be in some places only one layer. The kinetonuclei of the individuals in the deepest layer are anterior to the trophonuclei, while they are clear in the posterior end of the parasites of the superficial layer. The parasites of the intermediate layers have the kinetonuclei at the side of the trophonucleus (Fig. 18). These forms measure 2 to 3 μ wide and about 4 to 6 μ long, including the free flagellum when present. Passing posteriorly the intestine quickly narrows to a much smaller caliber where the parasites become smaller, measuring only 3 μ in length. In the rectum either these small pear-shaped forms or small spherical forms are found lining the wall in layers. Throughout the lumen of the intestine and rectum free forms (Fig. 16) and rosettes (Fig. 17) with flagella centrally directed are present. These pear-shaped forms or "gregarine" type are either pointed or truncated and the flagellum may or may not project beyond the end of the body (Figs. 15, 16). The side of the body along which the flagellum runs is thinner than the opposite side.

In a smear of the whole digestive tract, I chanced to obtain some of the "gregarine" type with very thick walls (Plate 5, Fig. 19). Some were spherical and very small, measuring only from 3 to 4 μ in diameter (Fig. 20). Others were pear-shaped or elliptical either free or in rosettes (Figs. 19, 21). The trophonucleus, kinetonucleus, and flagellum were plainly visible in the pear-shaped forms, but no flagellum could be found in the spherical individuals. In profile view the flagellum is seen to lie close to the wall. Chromatin granules were often found distributed in the cytoplasm. The wall appears under a magnification of 2,800 diameters to be perfectly homogeneous. Prowazek describes similar forms, but claims that the wall is beaded.

He says that they are cysts which are passed out with the feces and licked up by other flies, thereby causing a new infection.

Out of hundreds of ticks examined there was one exceptional condition found. In this case nearly all the parasites were rounded off, only a few of the typically flagellate forms being present. Occasionally a rounded form was met with among the flagellates of other ticks, but never in predominating numbers (Plate 4, Figs. 10, 11). All stages in the rounding-off process were present. The flagellate bends back on itself at about the middle point of the body (Plate 5, Fig. 28). The concave side thus formed apparently grows shorter and shorter till a rounded body is produced (Fig. 25). In other cases the parasite appears to roll up, the contiguous edges fusing (Figs. 30, 31). The flagellum often passes almost entirely around the body (Fig. 25). In others it curves around over the surface of the parasite and may end with the body wall or project outside as a free whip (Figs. 30, 31). The trophonucleus becomes less distinct while the kinetonucleus often nearly equals it in size. Such forms were pictured by Pfeiffer. Other flagellates in the same tick presented still more striking phenomena. In these the anterior end of the flagellum had curled back on itself. A very thin membrane was stretched across the loop to form a disc. The flagellum could be seen as a heavy line circumscribing the disc (Fig. 27). The first stage of this process is shown in Fig. 26. This same condition is said to occur in *Euglena.* In other forms the anterior end assumed the character of a sharp pseudopodium, while in still other cases the end was spread out like the plastic, lobose pseudopodia of some amebae (Fig. 29). These conditions were not the result of faulty technique, for they were observed in the living state and then killed over formol fumes and not smeared on the slide. Associated with these forms was the fact that the wall of the stomach was exceptionally tender. The round forms were marked by lightly staining areas and vacuoles. This fact together with apparent tendency toward nuclear degeneration might be taken as indications of their being involution forms.

Other round forms were found, whose shape later proved to be an artefact. They were present in sections of stomachs killed in Merkel's fluid. Invariably when this killer was used the flagellates crowded very tightly against and between the epithelial cells of the stomach and rounded off (Plate 4, Fig. 13). The flagellum was sometimes still visible where the parasites were not too close together. The kinetonucleus took a peripheral position perpendicular to the wall, which often became slightly thickened. The cytoplasm was clear and resisted staining. The killer which is weak evidently acted so slowly that the flagellates had time to react physiologically and take on those characters which are conducive to their power of resistance. At places where the epithelium was peeling off it was often hard to tell where the parasites left off and the cells began. In many cases I am sure the parasites were within the cells.

Features by which male, female, or indifferent forms could be distinguished were sought for in vain. With the work of Schaudinn (1904) and Prowazek (1904) in mind I spent a great deal of time in looking for forms that could possibly be interpreted as possessing different sex. To be sure in one tick were found large, more or less oval, or gregarine-like forms, which from their size and shape might be considered by the above authors to be females (Plate 4, Fig. 12). They have no more food material than the other forms nor any peculiarities about the nuclei. Some of the small forms may be males, but no evidence for considering them as such could be obtained. In fresh material two cases were found where a small form was clinging to the side of a large one near its posterior end. But at least three interpretations are possible. It may have been an agglutination, a division, or a conjugation of male and female.

The epithelium with the flagellates attached peels off from time to time, but not with the periodicity described by Pfeiffer. He says that the changes in the intestine and the infection of the same were quite similar to those given by Schaudinn for *Culex pipiens*, so that it was unnecessary for him to describe the process. Schaudinn claimed that the cuticular lining of the posterior part of the oesophagus becomes gelatinous and envelops the fresh blood as it passes through the oesophagus into the stomach. After this has taken place the parasites enter upon a period of multiplication. Toward the close of digestion they enter a resting stage and are found attached to the epithelial cells or between them. After the second feeding, and the fresh quantity of blood has become digested they enter upon a multiplication stage and then gradually collect in the anterior part, where the epithelium is regenerating. They now enter their second resting stage. When the proventricle of the oesophagus is withdrawn from the neck of the stomach the newly formed cuticula with the parasites attached is left behind in the stomach. As the fresh blood is introduced, this mass of parasites attached to the loose cuticle is carried back to the smallest portion of the intestine, where the wall ruptures and the parasites pass into the blood vessels. My observations do not support in any degree Pfeiffer's statement, to the effect that there are changes in the sheep-tick similar to those in *Culex pipiens*.

The tick's digestive tract and habits of feeding are very different from those of the mosquito. There is no proventricle present in the tick, while on the other hand the mosquito lacks a strong valve between the stomach and intestine. In the tick the valve is so efficient that no large masses of material can pass through it. In respect to feeding habits, the mosquito takes a meal and then goes for days or even weeks before getting another, or at least till the blood is digested. Thus there is an alternation between a condition of undigested and of thoroughly digested blood. These conditions do not occur in the tick which spends its whole life on the sheep, both fresh and digested blood always being present in the stomach in the natural state. All ticks have fresh blood in their stomachs when first removed from the sheep. Thus, on a-priori grounds alone, one would be led to the conclusion that these parasites would not behave the same as Schaudinn's. There has been entirely too much generalization by investigators who were anxious to ascribe to all flagellates the stages of life cycle described by Schaudinn. But, sure of so little of the life cycle of the flagellates as we are, one is not warranted in generalizing, merely for a-priori reasons, if for no others.

To be sure, in the sheep-tick portions of the epithelium peel off from time to time and with the attached flagellates pass along posteriorly with the stomach contents. But I can find no alternation between periods of rest and of multiplication. All the forms described, with the two exceptions mentioned, are to be found at the same time in the same tick whether the tick be filled with fresh blood or starved for over a week. Those very long lance-shaped forms (Plate 3, Figs. 2, 3, 4) were found in ticks, one of which was starved 18 hours and the other seven days, yet they cannot be considered as resting forms. There are present the free and the attached forms. The gregarine type peculiar to the intestine is always present in all infected ticks. There are, of course, slight variations in the size and shape of the parasites in various ticks, but these variations are apparently not due to the presence or absence of fresh blood. The epithelium peels off in such a manner that the attached parasites are not rolled up in it. They are thus left so that they can detach and become free in the intestine. The flagellates never pass forward into the anterior third of the stomach whether it is filled with food material or empty. And so in no respect do I find these flagellates behaving like Schaudinn's.

Seasonal changes apparently have no effect upon the flagellates. My observations extended through two years over the months between September and June. In cold weather the ticks remain in the wool on the skin of the sheep, while in hot weather they are found crawling over the surface of the wool. Hence their temperature is probably not effected much by change of seasons.

As regards the method of transmission Pfeiffer made no statement. Presumably he did not examine the blood of the sheep for the parasite. Knowing that insects act as passive carriers, if not as intermediate hosts for trypanosomes, it seemed possible or even probable that these flagellates were sucked up by the tick with the sheep's blood. If such be the case, a very large percentage of sheep must harbor the parasite since practically every full-grown tick is infected. But examinations of the blood, both in the fresh condition and in stained preparations, always showed negative results.

Aware of the hypothesis that *Piroplasma* may have a flagellate stage in an insect host, it occurred to me that I might be dealing in the tick with the flagellate stage of a piroplasma-like form in the sheep, which I had overlooked. Knowing the results of Rogers' experiments on the Kala Azar parasite, I proceeded to make similar cultures of the sheep's blood, with a view to obtain flagellated organisms, provided the tick flagellate had a stage of its life history in the sheep. For a detailed description of these experiments reference can be made to my paper (1908). Suffice it here to say that all sorts of actively motile flagellates and ameboid forms developed, but they were later shown to be a normal element of the blood, namely, blood-platelets. It was shown conclusively that they have no relation to the tick flagellates, for they were readily obtained in cultures of the blood of the dog, rabbit, and man.

In that paper I threw some doubt upon the truth of the statement of several authors that *Piroplasma* developed into flagellates in sodium citrate culture. It is certain, however, according to Rogers (1907) and Patton (1908) that the parasite of Kala Azar has a flagellate stage. In cultures Rogers obtained rosettes of flagellates exactly comparable to rosettes of *Herpetomonas* and *Crithidia* found in insects. Patton has found further that this parasite develops naturally in the stomach of the bed-bug into a true flagellate, very similar to insect flagellates. On the other hand, there are insect flagellates which probably never enter the blood of a vertebrate, but are transmitted directly from insect to insect. For example the common house-fly, which is not a blood-sucking insect, harbors a *herpetomonas* which, according to Prowazek, is transmitted through the egg and feces. Furthermore, Novy, MacNeal, and Torrey have performed inoculation experiments to ascertain whether flagellates found in mosquitoes have a vertebrate host also. They inoculated the flagellates into sparrows, canaries, pigeons, doves, hawks, owls, crows, rats, and mice, but always with negative results. These experiments, however, as they remark, do not prove conclusively that these parasites do not have a vertebrate host, for there may be some susceptible vertebrate which was not tried. They also found that mammalian trypanosomes soon perish in the stomach of insects, as is shown by the fact that inoculations into fresh animals of the stomach contents of insects a few hours after they have sucked infected blood give negative results. They have further shown that insects do not afford as favorable conditions for the multiplication of mammalian trypanosomes as culture media do. In a letter from Patton I am informed that he failed to trace any development in fleas or lice fed on rats infected with *Tr. lewisi;* also, in fleas and lice fed on a squirrel harboring *Tr. indicum.* He (1907) states further that the *Herpetomonas* which he found in *Culex pipiens* and the *Crithidia* of the water-bug (*Homoptera*) are peculiar to the insects, having "no connection with any blood parasite." He has also made the suggestion to me that the forms I described (1907) in the rat flea have no connection with the rat trypanosome, but are strictly insect flagellates. Balfour's discovery (1906) of a herpetomonadine flagellate in the flea (*Pulex cleopatrae*) and also Patton's discovery of one in the cat flea (*Ctenocephalus felis*) together with my own researches on the tick flagellates lead me to a tentative acceptance of Patton's suggestion.

It has already been stated that the flagellates of the sheep-tick are never found in the fresh blood of the anterior portion of the stomach. This in itself would indicate that the living blood of the sheep is not a favorable habitat for the parasites. Some experiments were made to ascertain whether the flagellates are acquired from the sheep's blood. Pupae of the tick were hatched out and the young ticks thus obtained were fed on rabbit's, sheep's or man's blood. Generally they will not suck for two to four days after hatching. The following table shows the results of these experiments:

TABLE 1.

	Tick	Date of Feeding	Died	Time of Examination	Flagellates Present
Rabbit's Blood	1	Nov. 6	Nov. 9	Nov. 9	None
	2	Nov. 10		28 hrs.	"
	3	Nov. 10		28 hrs.	"
	4	Nov. 10		68 hrs.	"
	5	Nov. 10		24 hrs.	"
Human Blood	6	Oct. 19, Oct. 29	Oct. 31	Oct. 31	"
	7	Oct. 24	Oct. 30	Oct. 30	"
	8	Oct. 28, Nov. 6		Nov. 9	"
	9	Mar. 28, Apr. 1, Apr. 8, Apr. 13, Apr. 15		Apr. 16	"
	10	Apr. 3, Apr. 5, Apr. 13	Apr. 18	" 18	"
	11	Apr. 1, Apr. 11		" 16	"
	12	Apr. 1		" 13	"
	13	Apr. 2		" 13	"
Sheep's Blood	14	Jan. 4, Jan. 5		Jan. 7	"
	15	Feb. 1, Feb. 4		Feb. 7	"
	16	May 13, May 17	May 21	May 21	Present
	17	Mar. 25	Mar. 30	Mar. 30	None

It will be noticed that only one tick developed flagellates. This one had been fed twice on sheep's blood and died within eight days after the first feeding, while some others were fed on human blood, in one instance as many as five times and kept for 19 days, yet possessed no flagellates. At first thought one might think that the data given in this table indicate that the flagellates were obtained from the sheep. But this is not the case. The blood of the rabbit and of man is not a natural food for the tick, and cannot be digested properly by it.

Although the blood is slowly changed and loses its structure, yet it is not digested like sheep's blood as is evidenced by the fact that it becomes hard and caked. Under such conditions it is reasonable to suppose that there is not afforded the nutritive stimulus necessary to bring about a development of the flagellates even though they might be present in the tick. And furthermore, these experiments are not extensive enough to be of conclusive value. Owing to the difficulty in keeping the ticks alive when removed from the host and on account of losing them accidentally much time was consumed in getting the scanty results shown in the table. It is needless to say that even though these experiments had been multiplied indefinitely they would not have given absolute proof on either side of the question. Stronger proof could have been adduced by the use of a fresh-born lamb which had never been bitten by a tick, but such a lamb was not available. If in using such a lamb the ticks should develop flagellates, the evidence would show that they obtained the flagellates from parent ticks but would not prove that the flagellate might not be carried into the blood of a sheep by the bite of an infected tick.

Practically all except the very young ticks were infected. There were, however, a few exceptions. Out of the hundreds of ticks examined, 18 were found uninfected and 16 of these occurred on a single sheep together with seven infected ticks. These facts could hardly be explained if the flagellates are acquired from the sheep. But the reasonable explanation is that these 16 ticks were the progeny of a female which had escaped infection, while the other seven came from infected females. From the negative evidence which has been adduced we are justified in concluding that the sheep does not act as a host for this flagellate.

If this conclusion is correct the method of transmission must be limited to fecal matter or the egg. While infection might be accomplished by a tick taking in some infected fecal matter on the skin of the sheep, it would necessarily be accidental and would rarely take place. The high percentage of infections could hardly be accounted for in this way. Examination of freshly voided feces occasionally showed lively flagellates. But after the feces had dried, although they were placed in salt solution several days, no signs of flagellates, neither of the monadine nor of the gregarine type, were seen. It is conceivable, however, that forms with a thick wall (Plate 5, Figs. 19–21) might live for a long time even under drying influences and retain their power of infection. With no definite evidence supporting infection through the feces, I believe the only natural mode of infection is transmission through the egg.

The female sheep-tick has two ovaries located laterally in the abdomen, the ducts of which unite to form a common, median oviduct. At the juncture of these ducts there is an enlarged chamber which functions as a sperm receptacle and is generally filled with sperm cells. The other end of the oviduct passes into a dilated portion, which serves as a uterus. The vagina passes from the uterus to the exterior. Milk glands open into the anterior end of the uterus. An egg matures in one of the ovaries, passes out, and after fertilization undergoes partial development in the uterus. The mouth of the developing embryo is near the opening of the milk glands, so that the milk may be sucked in. It might be possible for the young tick to suck up parasites along with the milk, but such a hypothesis was not supported by observation, parasites never having been found in the milk glands. While this embryo is developing in the uterus, an egg in the opposite ovary is maturing and will pass out soon after the expulsion of the embryo. This description of the reproductive system is sufficient for our purposes. The details of the embryology have been worked out by Pratt (1899).

Sections and smears of the ovaries were made with a view of finding the parasites in them. Prowazek was able to demonstrate parasites in the eggs of flies infected with *Herpetomonas*. According to him they are present at first in the copious vitellus where they can be demonstrated only in smear preparations. I have not been able to find the parasites in smear preparations of ova from the sheep-tick. Nor was it possible at first to find them in sections of ovaries killed in Zenker's fluid, which gave the best results for the intestinal forms. After killing with this fluid, the yolk material stains black and obscures the parasites. However, I was always able to find parasites in sections killed with Hermann's fluid and stained with iron hematoxylin. After finding them in these preparations I was able to locate them, though with difficulty, in material killed with Zenker's fluid. Spherical masses, measuring about 3 μ, with a chromatin body lying in a clear space were commonly found (Plate 5, Fig. 24). It should be noted that these are about the size of the small round forms of the intestine. From these are evidently formed by growth and by divisions of the trophonucleus larger masses with several similar chromatin bodies (Figs. 22, 23). Several of the round forms may be grouped together in irregular masses. They are always very distinct from the cytoplasm and have a very definite structure. They bear a strong resemblance to some of the forms found by Prowazek in the vitellus of the fly's egg (see Prowazek, 1905, Fig. 6, *d*). In these, he says, the nutritive nucleus perishes, while the kinetonucleus undergoes numerous divisions. He believes, therefore, that these forms perish, as he was not able to trace any further development of them. He figures the kinetonuclei as round or rod-shaped bodies lying in clear spaces. In many of my forms the chromatin bodies, which are very definite, vary from minute round granules to large, oval, or round masses. In one form (Fig. 22) there were indistinct, rodlike bodies at the edge and on the surface of the mass which might be interpreted as kinetonuclei. I am more inclined to believe that they were artefacts, little wrinkles in the surface, for they were not found in other forms. I cannot say whether the other chromatin bodies are trophonuclei or kinetonuclei. Possibly the larger ones are trophonuclei, the smaller ones kinetonuclei. When the forms represented by Figs. 22 and 23 were greatly destained the large chromatin bodies appeared as mere granules like the central granule in the trophonuclei of intestinal forms. And so, contrary to Prowazek, I am of the opinion that the large masses are trophonuclei. There can, however, be little doubt that these foreign masses are parasites which infect the progeny. The egg requires about a month for its development, and hence it is exposed for a long period to the possibility of infection. This perhaps explains why a tick is seldom found without infection. The further development of these forms I have not followed. Neither by sectioning nor by teasing the viscera in salt solution, was I able to find the parasites in freshly hatched ticks.

There are various opinions as to what characters belong to the genera *Crithidia* and *Herpetomonas* respectively. Léger (1902) described a flagellate occurring in the region of the malpighian tubes in the gut of *Anopheles maculipennis* for which he formed a new genus, *Crithidia.* These forms are small, measuring from 3 to 10 μ in length. The kinetonucleus is anterior to the trophonucleus and in the longer forms a rudimentary undulating membrane is present. The shorter forms are truncated and have no undulating membrane. In 1904 he described a flagellate occurring in the Tabanidae under the name *Herpetomonas subulata.* In this case he recognized both the long, slender and the short, pear-shaped, or truncated type. Here again the long forms, several times longer than *Crithidia*, have an undulating membrane, formed by the body being drawn out along the flagellum. The membrane is more marked than in his *Crithidia.* The short forms are found attached in layers to the wall of the intestine in the region of the malpighian tubes.

Patton (1907) described a flagellate in the water-bug under the name *Crithidia.* The monadine form resembles very closely the monadine forms of Léger's *Herpetomonas subulata.* In the same paper he figures under the name *Herpetomonas* a flagellate in *Culex pipiens*, the monadine form of which has a close resemblance to the monadine form of *Herpetomonas jaculum*, described by Léger (1902). These forms have no undulating membrane, and the kinetonucleus is near the anterior end. It will be seen, therefore, in comparing these forms, as has already been pointed out by Léger himself and by Novy, together with his associate workers, that the distinctions between the two genera are at best fragile. The forms described in this paper only add to the fragility of distinction.

Prowazek (1904) places in the genus *Herpetomonas* two species of flagellates, one from the gut of the common house-fly *Musca domestica*, the other from the meat-fly *Sarcophoga haemorrhoidalis.* In these forms he finds two terminal flagella with a delicate membrane stretched between them. They take origin in the kinetonucleus which is located in the anterior end of the parasite, and from which a double, twisted thread passes to the posterior end where it ends in a double granule, *diplosome.*

Lühe (1906) holds that the difference in size between the short crithidian type and the long herpetomonadine form does not justify the formation of two genera. He would, however, retain both genera and characterize *Crithidia* as possessing a kinetonucleus near the trophonucleus, a single flagellum, and a slightly developed undulating membrane, while *Herpetomonas* would be characterized by a terminal kinetonucleus and a double flagellum.

Novy, MacNeal, and Torrey believe that Léger in the case of *Herpetomonas subulata* was dealing with both genera, the small, pear-shaped, truncated forms belonging to the genus *Crithidia*, and the long forms to *Herpetomonas*. Furthermore, the *Crithidia minuta* which Léger later placed in the genus *Herpetomonas* because of the presence of a long form, they believe to be a true *Crithidia*. Their reason for this belief is that in cultures *Crithidia* and *Herpetomonas* have distinct characters and always retain them, never changing from one into the other. They further refuse to accept Lühe's classification until it has been confirmed that the *Herpetomonas* described by Prowazek always possesses a double flagellum. While they regard these two genera as representing primitive trypanosomes and hence to be placed in the genus *Trypanosoma*, yet, as long as the genera are retained, they would characterize them in the following way:

> "*The genus* Crithidia, *if it is to be retained, is characterized by peculiar, short oval or pyriform bodies which are usually round or obtuse posteriorly, while the anterior end is truncated, or even slightly depressed, and bears a short, straight flagellum. In this short form the nucleus is near the posterior end, and the blepharoplast is by its side. Somewhat longer cylindrical forms, tapering very slightly, or rounded at each end, may be present, and these are provided with a long flagellum. In this form the nucleus is near the center, and the centrosome is usually about midway between the nucleus and the anterior end. Both forms may occur in rosettes with flagella directed centrally. The undulating membrane and posterior diplosome, as seen in the* Herpetomonas, *are absent.*"

This characterization is based upon the results obtained from cultures, in which crithidian and herpetomonadine forms, as characterized above, retained respectively those characters throughout numerous sub-cultures. These authors claim to have found that mosquitoes may be infected either with *Crithidia* or *Herpetomonas*, or with both genera at the same time.

It is clear that the flagellates of the sheep-tick could be put under neither genus according to these authors' characterization. Their *Herpetomonas* has an undulating membrane and a diplosome. The longer forms from the sheep-tick have a distinct undulating membrane but no diplosome. *Herpetomonas*, they say, gradually tapers posteriorly. These forms, as I have stated before, are typically blunt at the posterior end. The short pear-shaped forms would go all right under *Crithidia*, but the longer monadine forms would not, since they possess an undulating membrane. If I am dealing with two genera, as they believe concerning Léger, then the short pear-shaped forms occurring in the intestine could be placed under the genus *Crithidia*, and for the longer type occurring in the stomach anterior to the malpighian tubes, a new genus would have to be created. But, according to Lühe, all these forms could be put in the genus *Crithidia*. It is very improbable that there are two different genera present. All gradations from the very long flagellate with a conspicuous membrane down to the small form with obscure membrane are to be found. Going from the anterior toward the posterior part of the stomach one can see in a general way a gradual reduction in the length and size of the forms. Passing through the valve into the intestine one comes to the true crithidian type. Here and there among the truncated forms attached to the wall of the intestine will be found forms that are intermediate between the truncated type and the more typically herpetomonadine forms found in the stomach. Rosettes in the intestine, as they are forming, are seen to be made up of individuals representing all stages from the purely herpetomonadine type down to the small crithidian form. While the latter type is never found in the stomach, yet the connecting forms are sufficiently numerous to establish their generic identity. And further, if the forms in the intestine belong to a different species from those in the stomach, it would be hard to account for the fact that both species are always associated. According to the laws of chance one would expect that occasionally only one of the species would be found. On the contrary, either both are present or both absent. Considering them as belonging to the same species these facts could, however, be accounted for on physiological grounds. Owing to the different degrees to which digestion and absorption have proceeded at successive levels along the digestive tract, one would expect to find certain differences in the morphology of the parasites correlated with these physiological differences. At the valve separating the stomach and intestine there is necessarily a much more marked physiological change than anywhere else. The end of the stomach narrows down to a very small caliber while the end of the intestine just on the other side of the valve is comparatively large and funnel-shaped. These marked anatomical changes must produce just as strong physiological differences, which are capable of modifying the flagellates as they pass from the stomach into the intestine. The crithidian type in the intestine is not found anterior to the valve because it would be mechanically impossible for them to pass anteriorly through it. The herpetomonadine type is found in the intestine because of the natural passage of stomach contents into the intestine. The parasites that are not passed out directly with the feces are doubtless modified into the crithidian type. Hence I conclude that only one species is present and that it belongs to the genus *Crithidia*, provided this genus is to be retained as characterized by Lühe. Mention has already been made of the fact that Patton has placed a very similar flagellate in the same genus.

As a matter of fact the distinctions between these genera are not sufficiently strong to receive generic rank but should rather be considered as specific. This form from the sheep-tick is intermediate between the two genera. In the possession of a very marked undulating membrane, this form unquestionably stands in close relation to the trypanosomes. By means of the cultural method Novy and his associates have pointed out the close affinities between insect flagellates and trypanosomes, contending that the former are primitive trypanosomes and that in cultures the trypanosomes revert in structure to the more primitive form. They would go so far as to place them all in the genus *Trypanosoma.* Granting, as I do, that they are correct in their contention that insect flagellates represent the primitive type of trypanosomes, it still seems to me that the modifications of the trypanosomes in structure and behavior, due to the marked change in habitat on account of being introduced into the blood of vertebrates, are strong enough differences to receive generic rank. The grounds for final retention or rejection of the genera *Crithidia* and *Herpetomonas* must be sought in their life histories. The genera cannot be intelligently characterized until the life histories have been followed out. If but one genus, *Trypanosoma*, is to be retained, a much closer agreement in the life cycles of insect flagellates and trypanosomes must be found, than is indicated by the knowledge we already have of their life histories. According to our present knowledge there are certainly much greater differences between trypanosomes and insect flagellates, than exist among the various species of insect flagellates or of trypanosomes.

If we accept the view that insect flagellates are the ancestors of the trypanosomes, it is easy to explain the origin of the undulating membrane. In respect to the trypanosomes the belief has been held that the membrane was formed by the fusion with the body of a second flagellum which was left to drag behind. Such an assumption is entirely unwarranted and is not supported by any morphological evidence in the trypanosomes. There is no indication of there being at present or of there ever having been a second flagellum. On the other hand, if we take a flagellate without a membrane, like *Herpetomonas jaculum* represented by Léger (1902, Fig. 12), as the primitive type, we can find all stages of development between this and the trypanosomes. In this form the kinetonucleus is near the end of the body and hence there is no reason for the existence of a membrane. Owing to the vibrations of the flagellum which must be transmitted more or less to the anterior portion of the body, there would be a tendency for the end of the body to become more flexible and membranous. Suppose the kinetonucleus be carried farther back as in the crithidian type, this effect would be intensified so that quite a definite membrane bordered by the flagellum would be produced. Now suppose the kinetonucleus be carried still farther back even posterior to the trophonucleus as in the trypanosomes, then it is clear that a strongly developed membrane would be formed along the body as far back as the point at which the flagellum passes into the body. Ontogenetically the position of the kinetonucleus is variable. In cultures of the trypanosomes it is found to pass from the posterior region to the anterior. Moreover, the membrane and flagellum are unstable, being entirely lost at times. Since such ontogenetic changes as these occur, it is not presumptuous to suppose that such phylogenetic changes as outlined above may have taken place. The changes are, therefore, to be regarded as progressive from a form having a single flagellum attached to the anterior end up to the trypanosomes which possess a well-developed undulating membrane bordered by a single flagellum. With their introduction into the blood, the mechanical consistency of their new habitat necessitated the development of a strong locomotor apparatus such as is now found in the blood trypanosomes.

My sincere thanks are due to Dr. Henry B. Ward for his direction

of this investigation which was carried on in the zoölogical laboratory of the University of Nebraska. His continued suggestions and criticisms have been very helpful in the work. I am also indebted to Dr. Joseph H. Powers for valuable suggestions.

Since the writing of this paper an extensive article by Minchin (1908) has appeared. He discusses the development of *Trypanosoma gambiense* and *Tr. grayi* in *Glossina palpalis.* Among *Tr. grayi* he found herpetomonas-like forms, which were without undulating membrane and had a kinetonucleus anterior to the trophonucleus. A comparison of his figures (Plate 11, Figs. 162, 164, 169, and 170) with mine (Plate 3, Figs. 1, 2, 3, and 4) will be sufficient to convince one of the marked similarity in structure. The formation of cysts in the proctodaeum as he describes and figures it (Plate 12, Figs. 186–200) is precisely similar to the process in the sheep-tick. He at first considered *Tr. gambiense* and *Tr. grayi* as identical but later came to regard them as distinct species. When this point was reached, he naturally agreed with Novy that *Tr. grayi* is a parasite of the fly. But after further reflection he has come to regard it as a parasite having a vertebrate host, probably, avian. I believe, nevertheless, that these herpetomonas-like forms, which are so similar to my *Crithidia* that is peculiar to the insect, *Melophagus ovinus,* may be insect flagellates and have no connection with the trypanosomes.

I have never been able to see a granule (blepharoplast, according to Minchin) at the base of the flagellum and separate from the kinetonucleus. In some cases the flagellum stains right up to the kinetonucleus, in others a portion remains unstained.

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EXPLANATION OF PLATES 3, 4, and 5.

FIG. 1.—Large monadine type from stomach of tick 40 hours after removal from sheep. ×2800.

FIG. 2.—A typical monadine form from the stomach of tick 68 hours after removal from sheep. Seven chromatin granules and a central granule are apparent. ×2800.

FIG. 3.—Monadine form from stomach of tick starved seven days. ×2800.

FIG. 4.—Lance-shaped monadine type from stomach of tick starved seven days. Posterior end sharply pointed. ×2800.

FIG. 5.—Short, thick, monadine form from stomach of tick 40 hours after removal from sheep. ×2800.

FIG. 6.—Short monadine type from stomach of tick 52 hours after removal from sheep. The trophonucleus possesses 11 chromatin granules. ×2800.

FIG. 7.—Monadine type from stomach of tick 72 hours after removal from sheep. Trophonucleus contains 11 chromatin masses, kinetonucleus dumb-bell shaped. ×2800.

FIG. 8.—Monadine type with posterior end contracted into a ball. From stomach of tick starved seven days. Four chromatin masses and central granule visible. ×2800.

FIG. 9.—Flagellate in process of division. From stomach of tick 26 hours after removal from sheep. ×2755.

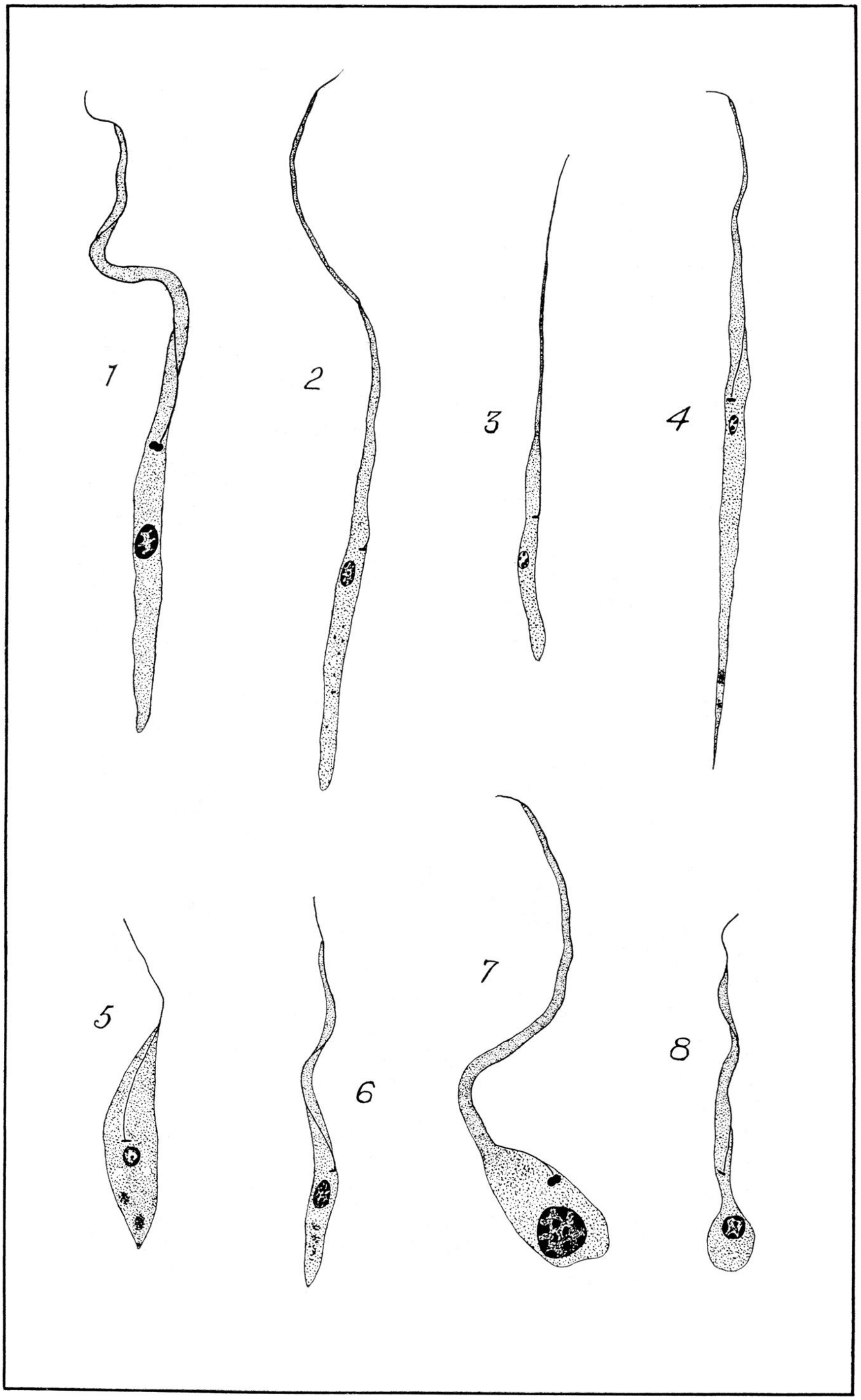
FIG. 10.—A round form with flagellum still present, from stomach of tick 52 hours after removal from sheep. ×2755.

FIG. 11.—Round form from the stomach of a tick starved three days. ×2755.

FIG. 12.—Female (?) from stomach of same tick as Fig. 10. ×2755.

FIG. 13.—Portion of cross-section of stomach of tick 40 hours after removal from sheep. Round parasites clinging to the epithelial lining. The form due to the effect of Merkel's killing fluid. ×650.

PLATE 3.



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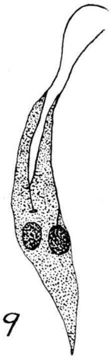
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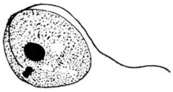
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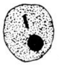
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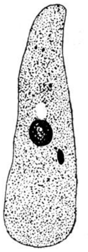
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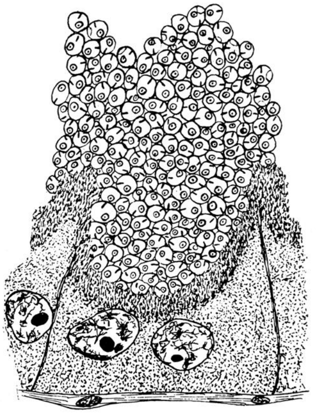
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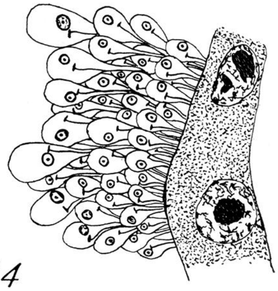
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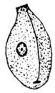
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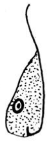
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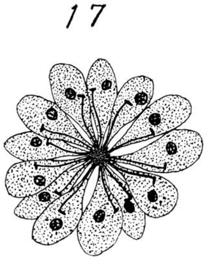
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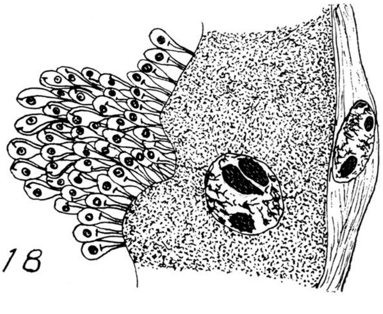
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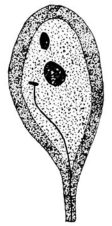
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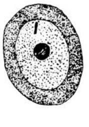
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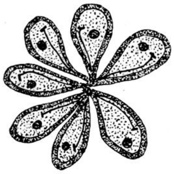
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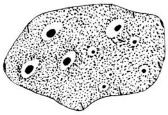
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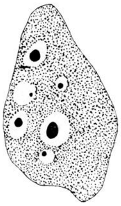
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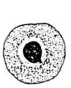
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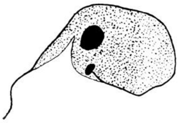
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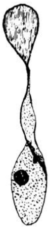
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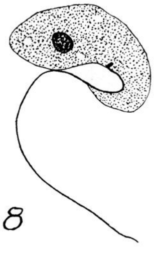
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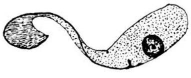
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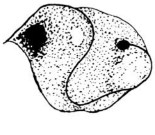

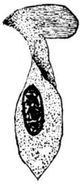
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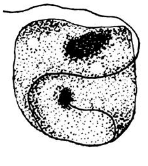
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FIG. 14.—Section through intestine cutting the posterior part of valve. Forms transitional between the long herpetomonadine type of the stomach and the short crithidian type of the intestine. ×1445.

FIG. 15.—Truncated form from the intestine of tick 12 hours after removal from sheep. ×2755.

FIG. 16.—Pear-shaped form with flagellum projecting beyond the body. From intestine of tick 18 hours after removal from sheep. ×2755.

FIG. 17.—Rosette of pear-shaped forms from the rectum of tick 18 hours after removal from sheep. ×2755.

FIG. 18.—From same section as Fig. 14. Parasites on the sides of the intestine. In this region the intestine is always dilated, its caliber being several times that of the posterior end of the stomach. The kinetonuclei of the basal layer are anterior to the trophonuclei, those of the outermost layer posterior to the trophonuclei, while those of the middle layers are intermediate between the two extremes. ×1445.

FIG. 19.—Pear-shaped cyst from tick 40 hours after removal from sheep, probably from intestine or rectum. ×2825.

FIG. 20.—Round cyst from same tick as Fig. 19. Flagellum absent. ×2825.

FIG. 21.—Rosette of cysts from same tick as Figs. 19 and 20. ×1180.

FIG. 22.—Parasite from the vitellus of the egg of the tick. Throughout the cytoplasm are clear areas containing chromatin bodies (probably trophonuclei). At the edge are rodlike structures (possibly kinetonuclei). ×3030.

FIG. 23.—Parasite similar to Fig. 22, but lacking the rodlike structures. From the same egg. ×3030.

FIG. 24.—Simple round parasite from another egg. In this one the nucleus was not so homogeneous in structure. An indefinite rodlike structure (kinetonucleus?) just above and partially to one side of the trophonucleus. ×2825.

FIGS. 25–31.—Peculiar involution (?) forms from the stomach of tick 40 hours after removal from sheep. Figs. 25 and 28 show early stages in the rounding-off process. Figs. 30 and 31 show the final result. Figs. 26, 27, and 29 show the plastic condition which the flagellum and anterior end of the body assume. In Fig. 26 the anterior end has bent back on itself and a membrane is stretched across the loop thus formed. In Fig. 27 this condition is carried still farther. In Fig. 29 the flagellum appears on one side as a definite line. ×2825.